only after an incubation period of 5 min. The potentiation is even stronger than that obtained with potassium cyanide. The contraction height is 3–6 times higher than the control instead of 1·9–3·7 times with cyanide, for potassium chloride concentrations ranging from 1 to 1·5 g per 1,000 (13·5–20 mM).

Fig. 1 curves describe muscle shortening (in millimetres) as a function of potassium chloride concentration in the bath, in normal conditions without any inhibitor, with cyanide and with fluoride.

Guinea-pig ileum. We observed that both enzymatic inhibitors previously used have the same influence on smooth muscle fibre potassium contracture as well as on skeletal muscle fibre.

Our experiments were performed on guinea-pig ileum, which is more sensitive than frog rectus abdominis: we obtained the same shortening with doses of potassium chloride 3–4 times weaker than in the previous experiments. Furthermore, the smooth muscle is more sensitive to the inhibitors, active doses of which did not exceed more than 1 per cent of those used on rectus abdominis. Also, it has been possible to reduce incubation period to 30 sec. So, in 0·03-mM potassium cyanide Tyrode, guinea-pig ileum shortening by potassium chloride is increased three-fold at a salt concentration less than 5·3 mM and two-fold at higher concentrations. At low potassium chloride concentrations the results are even more obvious with sodium fluoride, but at higher concentrations the potentiation is only 1·2–1·3 times.

We tried other enzymatic inhibitors with the same method: 2,4-dinitrophenol, sodium iodoacetate, sodium nitrite. No definite conclusion can be drawn from these later experiments because the results were irregular.

As a whole, the results reported here show that enzymatic inhibitors are able to sensitize smooth and striated muscle fibres to contracture produced by potassium chloride, by influencing either cellular oxidation phenomenon (cyanide) or glycolysis (fluoride).

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PHARMACOLOGY

A New Antiviral Agent: 4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone, M and B 7714

STIMULATED by reports of the antiviral activity of some thiosemicarbazones[1], and particularly of the activity against neurovaccinia of isatin β-thiosemicarbazone[2,3], we sought to enhance the activity of these compounds by structural modification. Thus we synthesized *N*-ethylisatin β-thiosemicarbazone independently of, but somewhat later than, Bauer and Sadler[4]. At the same time, we had been making a systematic study of the chemistry and chemotherapeutic properties of derivatives of the new monocyclic ring system, 1,2-thiazole (isothiazole), and we observed that 3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (M and B 7453) also protected mice infected intracerebrally with neurovaccinia. The relatively high toxicity of this compound (acute LD_{50} = 0·7 mg/g orally in mice) led us to examine related thiosemicarbazones, one of which, 4-bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (M and B 7714), is considerably less toxic (acute LD_{50} = 4·3 mg/g orally in mice).

4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone is a yellow crystalline solid, m.p. 228°–230° (decomp.)[5]. It is less than 0·1 per cent w/v soluble in water at 37°, but dissolves as a salt in alkaline solutions.

In mice infected intracerebrally with neurovaccinia (*IHD* strain) and dosed orally once daily for four days, a marked protection was observed (Table 1).

Table 1. THERAPEUTIC ACTIVITY OF M AND B ADMINISTERED ORALLY TO MICE INFECTED INTRACEREBRALLY WITH NEUROVACCINIA

	Daily dose mg/kg (× 4)	Mean survival time (days)	Animals surviving at 10 days
Controls	Nil	4·6	0/30
M and B 7714	500	9·4	22/30
M and B 7714	250	9·0	18/29
M and B 7714	120	8·0	12/29

Table 2. EFFECT OF DELAYED DOSAGE WITH M AND B 7714 ON THE SURVIVAL OF MICE INFECTED INTRACEREBRALLY WITH NEUROVACCINIA

	Dose mg/kg	Delay before treatment (days)	No. of doses given	Median survival times	Animals surviving at 14 days
Controls	Nil	–	–	4·8	0/10
M and B 7714	1,000	0	4	12·7	8/10
M and B 7714	1,000	1	3	13·2	9/10
M and B 7714	1,000	2	2	11·1	7/10
M and B 7714	1,000	3	1	8·4	4/10

Protection was still observed when treatment was delayed (Table 2). Surviving treated animals were immune to challenge from 10^5 LD_{50} infecting doses of virus. During treatment, the circulating virus titre was below that in the infected, untreated, controls. The degree of meningo-encephalitis was less after treatment.

The compound has been tested against variola major in baby mice and found to be active[6].

M and B 7714 also shows high activity in rabbits infected intranasally with rabbit pox (Utrecht strain), but is inactive against influenza, encephalomyocarditis (Columbia *SK*), and rift valley fever viruses.

Details of a fuller laboratory investigation, including toxicological studies, and the results of an extended therapeutic and prophylactic trial will be published in due course.

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Distribution of Homovanillic Acid in the Human Brain

HOMOVANILLIC acid (3-methoxy-4-hydroxyphenylacetic acid, HVA) is formed by the action of the enzymes monoamine oxidase and catechol-*O*-methyl-transferase as a final product of dopamine metabolism, and its occurrence in urine has been described[1–6]. From other investigations[7,8] it may be concluded that in experimental conditions the HVA level in the brain tissue reflects the dopamine turnover, although with a certain delay. It also seems likely that for the normal brain tissue a similar statement may be made. The actual HVA concentration in the normal brain tissue, therefore, might be a suitable index in the evaluation of the local dopamine turnover. Sharman[9] has recently demonstrated the presence of HVA in the caudate nucleus of animals of several species. This seems consistent with the high dopamine concentration in this brain area[10]. Detailed investigations have been performed on the local distribution of dopamine in the human brain[11–15]. This account describes the occurrence and local distribution of HVA in the human brain.

Human brains were selected in the autopsy room, from patients not having suffered from cerebral or mental

disease, 3–18 h after death. The brains were dissected immediately and the selected tissues were frozen and kept at −20° C until the extraction procedure took place, usually within two days. The HVA was extracted by Sharman's method[9] and the final extracts were subjected to a two-dimensional ascending paper chromatography on Whatman No. 1 paper in the following systems: isopropanol : water : concentrated ammonia solution in the proportions 40 : 9 : 1, and benzene : propionic acid : water in the proportions 100 : 70 : 4. Spots were detected by spraying the air-dried papers with freshly prepared alkaline solutions of diazotized sulphanilic acid or of diazotized *p*-nitroaniline[16]. The HVA was identified by its R_F-values and colour reactions, compared with reference spots of synthetic HVA. For the quantitative evaluation the sulphanilic acid spray only was used. The stained areas corresponding to HVA were cut out, put into test tubes, and eluted with 4 ml. of a mixture described by von Studnitz[17]. The optical density of the eluates was assayed at 500 mμ in a Beckman *DU* spectrophotometer. Standard curves were prepared from 2 to 16 μg of synthetic HVA, including the chromatographic procedure, and appropriate paper blanks were subtracted. Sufficient amounts of tissue were extracted to achieve distinct spots of HVA on the chromatogram. However, in some brain areas the tissue level of HVA was low and no colorimetric assay could be performed: in these areas the HVA concentration was estimated to be < 1·0 μg/g.

Table 1. Homovanillic Acid in Human Brain

Region	No. estimations	Homovanillic acid (μg/g tissue) Range	Mean
Caudate nucleus	3	1·3–2·4	1·8
Putamen	4	2·8–5·3	4·0
Globus pallidus	5	1·6–3·4	2·4
Substantia nigra	3	1·7–2·0	1·8
Hypothalamus	3	< 1·0	—
Thalamus	4	< 1·0	—
Nucleus amygdalae	1	< 1·0	—
Nucleus ruber	1	< 1·0	—
Floor of IVth ventricle (Formatio reticularis)	2	< 1·0	—
Cortex (Gyrus praecentralis)	2	< 1·0	—
White matter	2	< 1·0	—
Nucleus dentatus cerebelli	2	< 1·0	—

The results are summarized in Table 1. They show clearly that concentrations of HVA exceeding 1·0 μg/g were found in several regions of the human brain: the caudate nucleus (1·8 μg/g), the putamen (4·0 μg/g), the globus pallidus (2·4 μg/g) and the substantia nigra (1·8 μg/g). It is worth noting that all these areas belong to the extrapyramidal system. In the other areas investigated, namely, the thalamus, hypothalamus, nucleus ruber, nucleus amygdalae, floor of the IVth ventricle with the reticular formation, cortex (gyrus praecentralis), white matter and the dentate nucleus of the cerebellum, the HVA concentration was too low for accurate colorimetric assay and was considered to be < 1·0 μg/g.

It may be stated that in all brain areas where the HVA concentration is less than 1·0 μg/g the dopamine level is also low. Within the corpus striatum, on the other hand, the high HVA concentration (1·8 μg/g in the caudate nucleus and 4·0 μg/g in the putamen) seems to be related to the high dopamine concentration in this region. The concentration of HVA in the substantia nigra (1·8 μg/g) also seems to be consistent with a distinct dopamine accumulation in this area[15]. In contrast, no similar relation can be established for the globus pallidus, where a high HVA concentration (2·4 μg/g) was found, but where the dopamine concentration is low. This high concentration of HVA in the globus pallidus should not be disregarded, as it seems to indicate a high dopamine turnover in a place where little dopamine accumulation occurs. The significance of a high dopamine turnover in the globus pallidus cannot yet be explained, but it might be related to the fact that the major afferent fibres of the globus pallidus stem from the dopamine-rich corpus striatum.

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Semi-automatic Extraction using a Rotary Extractor

A simple rotary extraction procedure has been developed for the extraction of urine with chloroform. It has been found to be efficient for the extraction of unconjugated aldosterone from urine. It virtually obviates emulsion formation and has been adopted for routine use in this laboratory; it appears to be suitable for wide application in liquid–liquid extraction systems.

Two parallel, horizontal, rubber-padded metal rods are rotated by a motor through a reduction gear. A glass bottle containing urine and chloroform is laid on the rods, and partition of solute between the two liquid layers occurs as the bottle rotates on the rods (Fig. 1). In these experiments the glass bottles were of approximately 5·5 in. diameter, the rollers were 4 in. apart, and the bottles revolved 32 times per min; 10-min rotation periods were used for each extraction. At this speed the chloroform layer remains below the aqueous layer during the period of rotation and the weight of the liquid maintains the bottle in position on the rods without external support. Several bottles can be used simultaneously. The recovery of added labelled aldosterone was measured in 23 urine specimens from patients with a variety of diseases—precisely those circumstances in which the frequent occurrence of emulsions had previously occurred using separating funnels for extraction. When the daily urine volume was two or more litres, 1 l. was taken for extraction; otherwise the urine was diluted to 2 l. with water and 1 l. was used. After adjustment of the *p*H to 6·5, the urine was extracted five times with 250 c.c. re-distilled chloroform (containing 1 per cent ethanol, v/v, as stabilizer) to remove free aldosterone. The chloroform layer was withdrawn from the bottle by aspiration each time and discarded. The aqueous phase was then adjusted to *p*H 1·0 with hydrochloric acid, radiochemically pure 1,2-^{3}H aldosterone (0·1–0·2 μC) was added, and the mixture was allowed to stand at room temperature for 24 h with occasional shaking; this procedure is known to release free aldosterone from its acid-hydrolysable conjugate. After 24 h the urine was re-extracted with chloroform and the total volume of 1·25 l. chloroform was washed with small volumes of dilute sodium carbonate and water, taken to dryness and made